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THE EFFECT OF DENERVATION ON THE ACTION OF SYMPATHOMIMETIC AMINES ON THE NICTITATING MEMBRANE

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The work of Cannon and Rosenblueth and their collaborators on the hypersensitivity of denervated structures has given rise to a general impression that after the denervation of a tissue there is always an increased response to a chemical stimulus. However, there are notable exceptions to this rule even among the limited class of sympathomimetic amines acting on structures innervated by postganglionic sympathetic fibres. Burn and Tainter (1931) observed that the iris of the cat's eye was insensitive to the dilator action of tyramine and much less sensitive to that of ephedrine when the superior cervical ganglion was removed one or two weeks previously. On the iris of the rabbit Drake, John, Renshaw, and Thienes (1939) found that *p*-hydroxyamphetamine, which caused dilatation of the normal iris, failed to do so after denervation. Burn (1932) found that the constrictor action of tyramine and of ephedrine in the vessels of the cat's foreleg was diminished or abolished by removal of the stellate ganglion 2–3 weeks previously. These results suggested that the effect of tyramine was depressed by denervation in all tissues, but Bacq (1937) stated that this conclusion did not hold good for the nictitating membrane in the cat under Dial anaesthesia. Bülbring and Burn (1938) therefore investigated the action of tyramine in a series of cats and found that the most usual result was that the denervated membrane was more sensitive to low doses, but the normal membrane was more sensitive to high doses.

At the same time they investigated hydroxytyramine and neosynephrine (Sympatol). Their general conclusion was that the more the structure of the compound departed from that of adrenaline the more often the denervated membrane was found to respond less than the normal membrane.

While it was thus clear that substances such as tyramine and ephedrine did not stimulate denervated tissue in general as much as normal tissue, the change in the response of the nictitating membrane in the early period after the operation was still unknown. We have therefore studied this question in some detail with a wider range of substances, and have been able to show that sympathomimetic amines fall into three groups so far as their action on the denervated membrane is concerned.

METHOD

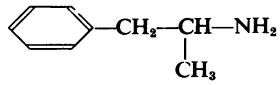
The right superior cervical ganglion was removed in an aseptic operation on each of 60 cats. After a varying interval each cat was anaesthetized with ether, and the spinal cord was divided by Dale's method (Burn, Finney, and Goodwin, 1950) without clamping the carotid arteries during the operation. The anaesthetic was discontinued and artificial respiration was given. The left cervical sympathetic chain was cut. The cat's head was then fixed rigidly and threads were passed through the nictitating membranes so that their contractions could be recorded by an isotonic lever which magnified the contractions 7.4 times. The blood pressure was recorded from one femoral artery and injections were made into the femoral vein of the opposite side.

RESULTS

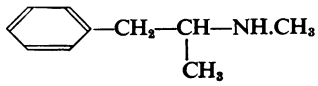
The sympathomimetic amines which were tested were found to be divisible into three groups according to their effects. The first group and the second group contained substances having only one -OH group in the ring or none ; the first group differed from the second group in that the second group contained substances having an -OH on the C atom next to the ring. The third group contained substances having two -OH groups in the ring ; it also contained the substance *m*-Sympatol or neosynephrine.

Compounds of the First Group.—The compounds of the first group which were examined were the following.

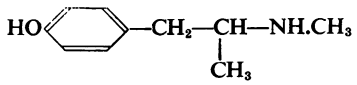
Amphetamine



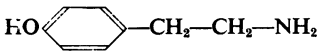
N-Methylamphetamine



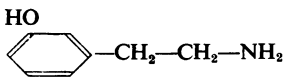
p-Hydroxy-N-methyl-amphetamine



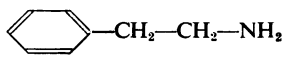
Tyramine



meta-Tyramine



β-Phenylethylamine



The action of the first three compounds in this group differed in one important respect from the action of the last three compounds. Each of the first three compounds possessed a $-CH_3$ group attached to the α-carbon atom, a grouping which prevents the destruction of these substances by amine oxidase. Their action was prolonged, and a succeeding dose was given when the previous dose had produced its maximum effect; each effect was measured from the original base line.

Early Effect.—The effect of denervation on the response of the membrane to substances of the first group changed according to the time which elapsed after removal of the ganglion. When observations were made on the first day after operation, that is, within 19–24 hours, the denervated membrane responded much more than the normal membrane, whatever the dose injected (Fig. 1). This effect was observed in 2 cats with N-methylamphetamine, in 1 cat with *p*-hydroxy-N-methylamphetamine, in 2 cats with tyramine, and in 1 cat with β-phenylethylamine.

On the second day after operation, from 30–40 hours, the response of the denervated membrane fell below the response of the normal membrane for all but low doses. This is illustrated in Fig. 2. This change was observed in 2 cats with N-methylamphetamine and in 1 cat with β-phenylethylamine.

Late Effect.—When substances of this first group were examined three days after removal of the ganglion or later, the response of the denervated membrane was as a rule found to be much less than that of the normal membrane as is shown for N-methylamphetamine in Fig. 2; this substance was tested in five cats between 4 and 48 days after operation, and the result was essentially the same in all of them. Similarly, when amphetamine was tested, the results on 4 cats examined 5–7 days after denervation were the same as those with N-methylamphetamine. There was only a very slight contraction of the denervated membrane which did not vary as the dose increased, while the contraction in the normal membrane grew in proportion to the dose. The mean response in the 4 cats to 1.6 mg. amphetamine was a contraction of 40 mm. in the normal membrane, and of 5 mm. in the denervated membrane.

The third substance of this group was *p*-hydroxy-N-methylamphetamine. In 5 cats examined 3–48 days after operation the response of the denervated membrane was much less than that of the normal membrane for all but low doses (see Fig. 3). The mean response in 5 cats to the low dose of 0.2 mg. was 6 mm. in the denervated membrane, and it was 1 mm. in the normal membrane. The response

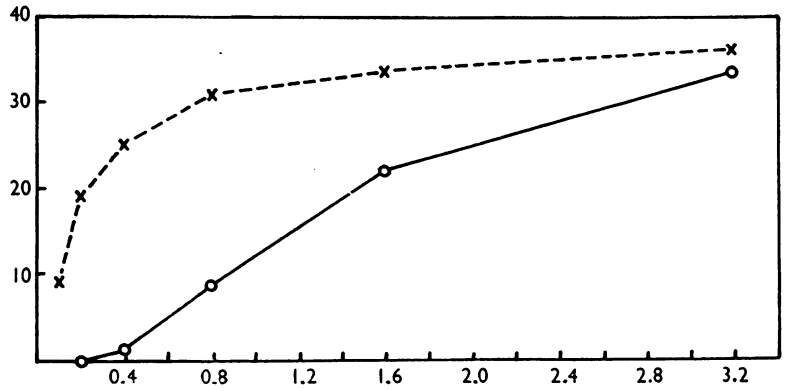


FIG. 1.—Response of nictitating membranes to N-methylamphetamine within 19–24 hours after unilateral excision of the superior cervical ganglion. Broken line is the response of the denervated membrane; full line is that of the normal membrane. Ordinates: height of contraction (mm., magnification × 7.4); abscissae: dose in mg. (mean of 2 cats).

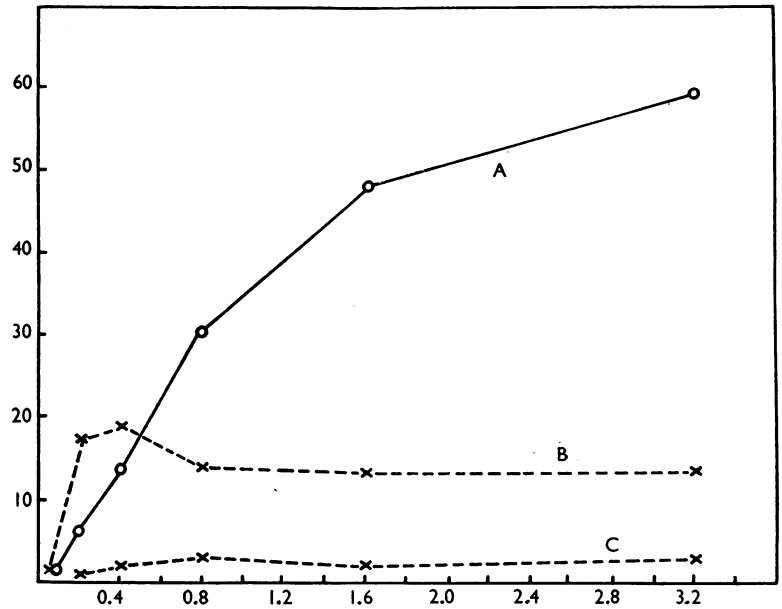


FIG. 2.—Response of nictitating membranes to N-methylamphetamine. *B* is the mean response of the denervated membrane in 2 cats, 30–40 hr. after unilateral excision of the superior cervical ganglion. *C* is the mean response in 5 cats, 4–48 days after excision of the ganglion. *A* is the response of the normal membrane in the 7 cats.

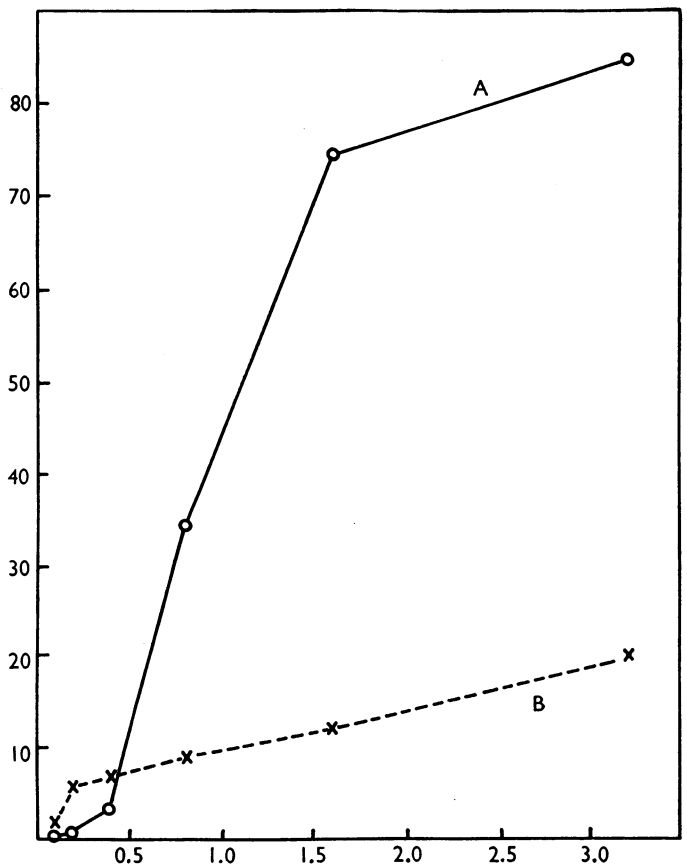


FIG. 3.—Mean response of nictitating membranes in 5 cats to *p*-hydroxy-N-methylamphetamine (pholedrine), from 3–48 days after unilateral excision of the superior cervical gang'ion. *A*, normal membrane; *B*, denervated membrane. Note that the introduction of the *p*-hydroxy-group results in more effect on the denervated membrane than that seen in Fig. 2 *C*, and resembles that in Fig. 2 *B*.

of the denervated membrane to *p*-hydroxy-N-methylamphetamine when larger doses were used, though much smaller than that in the normal membrane, was not so trivial as the response to amphetamine or to N-methylamphetamine, as a comparison of Fig. 2 and Fig. 3 shows. From the results so far described with substances of the first group, the effect of removing the ganglion was to increase the response of the membrane on the side of operation to all doses during the first 24 hours, then to diminish the response when high doses were used, and finally to diminish the response to all doses. This succession of changes was not uniform for all members of the group. With amphetamine and N-methylamphetamine the succession of changes was most complete; the *p*-hydroxy derivative of the latter compound, however, when injected in low doses persisted in causing more contraction in the denervated membrane. Thus a remnant of hypersensitivity survived to this substance in the denervated membrane.

Results with tyramine obtained from observations on 15 cats examined 5–48 days after operation suggested that there was a similar succession of changes in the response of the denervated membrane, but that the rate of change varied greatly. In 1 out of the 15 cats, the response of the membrane denervated for 7 days was greater than that of the normal membrane to all doses; in 10 of the cats the response of the denervated membrane was greater than that of the normal membrane to small

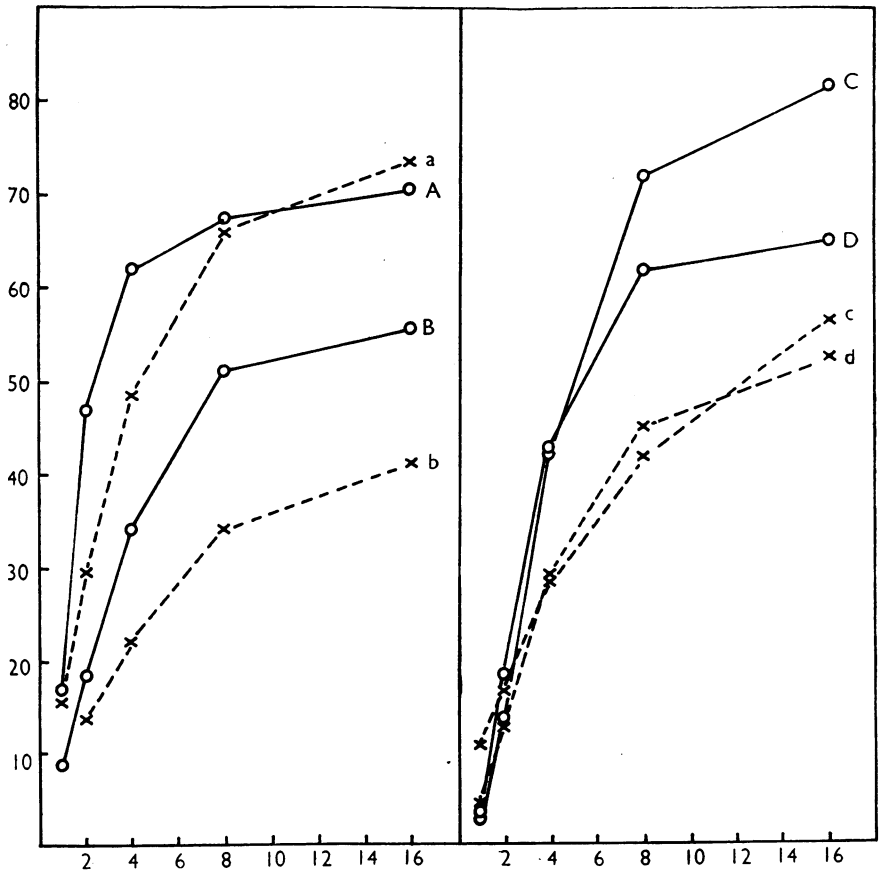


FIG. 4.—Results with substances having an —OH group on the carbon atom of the side chain next to the ring. *A* shows mean response of normal nictitating membrane to ephedrine in 6 cats; *a* shows corresponding response of membrane denervated 4–48 days. *B* shows mean response of normal membrane to β-phenylethanolamine in 4 cats; *b* shows response of membrane denervated 3–41 days. *C* shows response to *p*-hydroxy-β-phenylethanolamine in 5 cats; *c* shows response of membrane denervated 4–8 days. *D* shows response to *p*-hydroxyephedrine in 4 cats; *d* shows response of membrane denervated 4–8 days. Note that all these compounds have more action on the denervated membrane than those of the first group.

doses, but it was much smaller than that of the normal membrane to larger doses ; in the remaining 4 cats the response of the denervated membrane was always less than that of the normal membrane. These results are very similar to those described by Bülbring and Burn (1938). They also made observations on 15 cats, and instead of the number of cats in the above groups being 1, 10, and 4, they observed 2, 11, and 2 respectively.

Observations were made with *meta*-tyramine on one cat. The results were similar to those with tyramine ; the denervated membrane responded more to injections of 0.4 and 0.8 mg., but it responded less than the normal to injections of 1.6 and 3.2 mg.

Thus it appeared that the increased sensitiveness seen immediately after operation to all substances of this group could persist to tyramine perhaps indefinitely in a small proportion of cats (3 out of 30). However, in the majority of cats a change occurred in the denervated membrane within a few days after operation as a result of which there was no increase in the response of the denervated membrane with increase of dose, and the hypersensitivity was evident only for low doses.

The last substance in the group to be examined was β-phenylethylamine, which was tested in 5 cats from 3–44 days after operation. Here also the effect on the normal membrane was much greater than the effect on the denervated membrane. However, in 4 out of the 5 cats there was evidence that in response to the lowest doses the denervated nictitating membrane contracted more than the normal nictitating membrane.

Tyramine and β-phenylethylamine differed from amphetamine and N-methylamphetamine in the response of the normal membrane ; its response

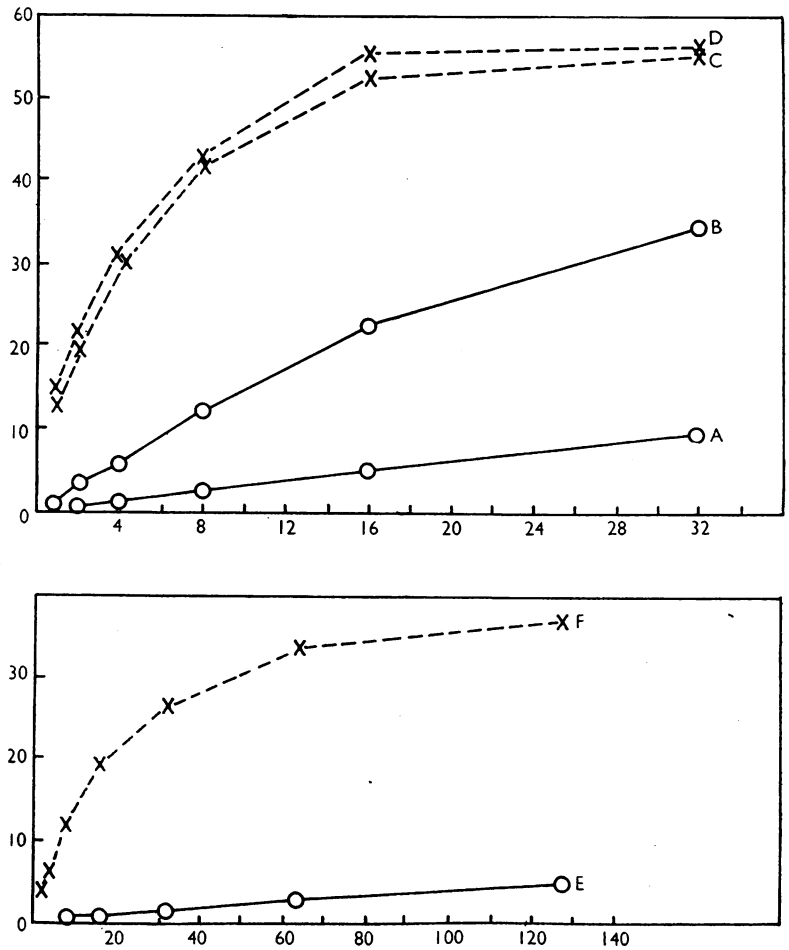
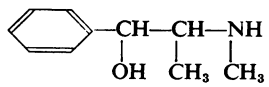


FIG. 5.—The upper chart shows the mean responses of nictitating membranes in 25 cats to noradrenaline and adrenaline. Superior cervical ganglion excised from 4–13 days. *A* is response of normal membrane to noradrenaline, *B* to adrenaline. *C* is response of denervated membrane to noradrenaline and *D* to adrenaline. The lower chart shows the mean responses in 6 cats to corbasil; ganglion excised 2–43 days. *E* is the normal response, *F* the response of the denervated membrane.

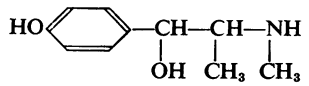
to the former substances was almost linear in relation to the dose.

Compounds of the Second Group.—The substances in the second group were substances containing an -OH group attached to the C atom next to the ring. They were:

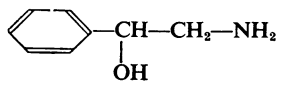
Ephedrine



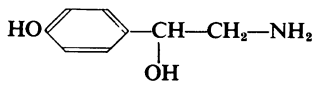
p-Hydroxyephedrine



β-Phenylethanolamine



p-Hydroxy-β-phenylethanolamine



The results obtained with these substances were similar to those of the first group in that these compounds were all active in causing contraction of the normal nictitating membrane. β-Phenylethanolamine and its *p*-hydroxy derivative both acted for a longer time than β-phenylethylamine and tyramine respectively. Again it was observed that the compounds with a $-CH_3$ group on the α-carbon atom exerted a much more prolonged effect than those without this substituent. The early effect of denervation within the first 24-hour period was to augment the response. This was observed in 4 cats with each of the substances in this group.

The later effect of the denervation was to diminish the response, though not to diminish it greatly. The mean results for all four compounds are shown in Fig. 4. *D* and *d* are the results of observations in 4 cats unilaterally denervated 4–8 days when *p*-hydroxyephedrine was injected. *C* and *c* are the similar results obtained in 5 cats denervated 4–8 days when *p*-hydroxy-β-phenylethanolamine was injected. *B* and *b* are results obtained in 4 cats denervated 3–41 days when β-phenylethanolamine was injected. With ephedrine the results were the same in 4 cats out of 6. In the other two examined respectively 7 and 9 days after operation, the denervated membrane still responded more than the normal membrane, the difference being greater when the higher doses were used. The mean results of all six cats, shown in *A* and *a* of Fig. 4, therefore indicate less difference between the response between the normal and denervated membranes than that for the other three compounds.

Since the denervated membrane continued to respond well, though less than the normal membrane, to substances of this second group for many weeks after denervation, it

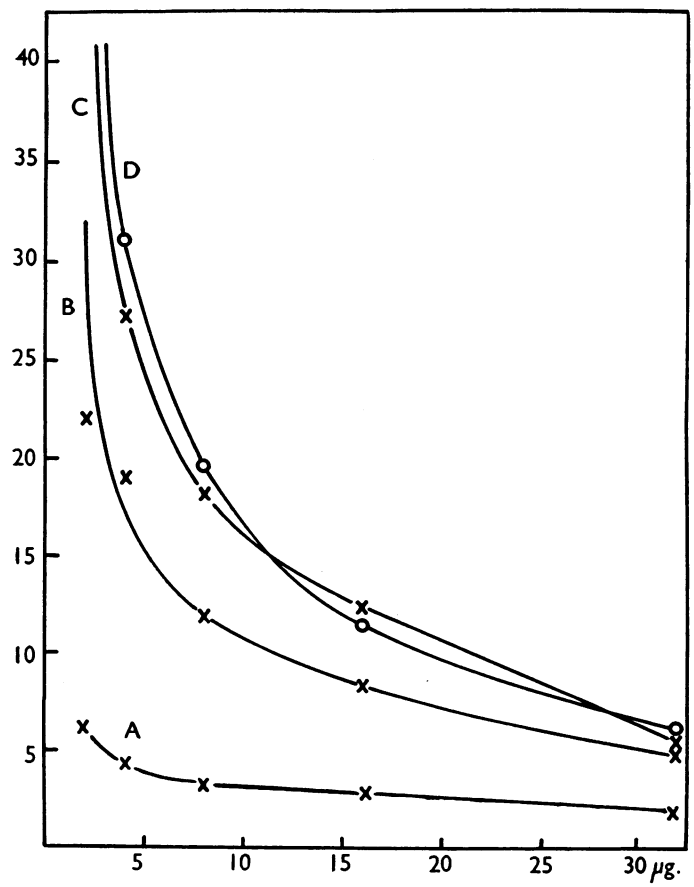


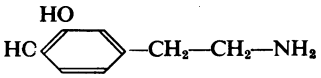
FIG. 6.—Ratios of contractions of denervated to normal nictitating membranes when noradrenaline is injected. Ordinate is ratio, abscissa is dose in μg. *A* mean result in 8 cats, 19–27 hr. denervated. *B* mean result in 6 cats, 30–66 hr. denervated. *C* mean result in 25 cats, 4–13 days denervated. *D* mean result in 8 cats, 37–50 days denervated.

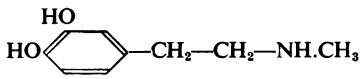
was evident that the presence of the -OH on the C atom next to the ring greatly increased the power to stimulate the denervated structure.

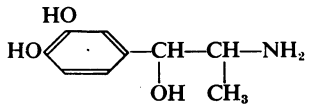
Compounds of the Third Group.—The compounds of the third group were, with one exception, compounds with two hydroxy groups in the ring in positions 3 and 4. The exception was neosynephrine, which is a substance having the same side chain as adrenaline attached to an *m*-hydroxyphenyl group. The compounds were:

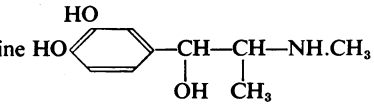
Noradrenaline

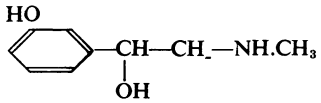
Adrenaline

Hydroxytyramine 

Epinine 

Corbasil 

Dihydroxyephedrine 

Neosynephrine 

The effect of denervation on the response to noradrenaline and to adrenaline is already well known. The denervated membrane becomes more sensitive and remains more sensitive indefinitely, though in a small proportion of cats Bülbring and Burn (1938) found a diminished sensitivity to large doses of adrenaline.

The effect of noradrenaline on the normal membrane is very small, and less than that of adrenaline. Nevertheless, the increase in sensitivity after removal of the ganglion was found to develop much more slowly for noradrenaline than it did for the substances in the two groups already considered. While the sensitivity of the denervated membrane was greatest at 19–24 hours for the substances in the first and second groups, the sensitivity for noradrenaline at this time was not much greater than that of the normal membrane, and on the average it was not maximal until somewhere between the third and fourth day. The increase in the sensitivity of the denervated membrane to adrenaline proceeded at a faster rate than that to noradrenaline, but by the fourth day the average sensitivity of the denervated membrane was the same for both substances (Fig. 5).

The increase in the sensitivity to noradrenaline after denervation is shown in Fig. 6, in which the ratio of the contraction in the denervated membrane to the contraction in the normal membrane (the D : N ratio) is plotted against the dose. The different curves represent the mean result at various times after operation. The mean curve for cats examined from the 4th–13th day was found to be practically the same as that for cats from the 37th–50th day.

Our observations indicated that the rate of increase of sensitivity for noradrenaline was similar to that for corbasil, epinine, and dihydroxyephedrine. The similarity between corbasil and noradrenaline is evident from Fig. 5. When the D : N ratio was calculated for each of these substances and was plotted against the logarithm of the dose expressed in moles, curves approximately parallel were obtained for the four substances, as shown in Fig. 7.

Neosynephrine (*m*-Sympatol) was found to affect the nictitating membranes in the same way as adrenaline.

The similarity between the relative responses of the nictitating membranes to noradrenaline and to corbasil is of interest because the latter substance possesses a $-CH_3$ group on the α-carbon atom whereas the former does not. Corbasil is not destroyed by amine oxidase as is noradrenaline, and indeed recent observations in this laboratory by Miss J. A. Robinson have shown that the presence of corbasil does not inhibit the action of amine oxidase on either *iso*amylamine, tyramine, or adrenaline.

The action of corbasil and of dihydroxyephedrine is also peculiar, because, although both these substances contain a $-CH_3$ group attached to the α-carbon atom, their effect on the nictitating membrane is similar in duration to that of noradrenaline and dissimilar from the prolonged effect of amphetamine or ephedrine.

The different substances in this group while similar in the above respects were dissimilar in activity. Corbasil had about one-fifth, epinine about one-tenth, dihydroxyephedrine about one-twenty-fifth, and hydroxytyramine about one-fiftieth the pressor activity of noradrenaline in the spinal cat. The injection of 80 μg. corbasil, however, caused a mean contraction of the denervated membrane of only 34 mm. in 6 cats as compared

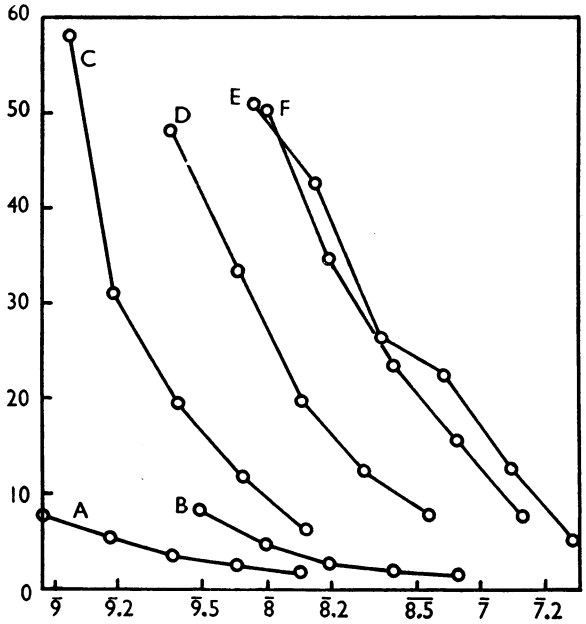


FIG. 7.—Ordinate is ratio of contractions in denervated and normal membranes. Abscissa is logarithm of dose in moles. *A:* adrenaline; mean of 25 cats. *B:* neosynephrine (*m*-Sympatol); 4 cats. *C:* noradrenaline; 25 cats. *D:* corbasil; 6 cats. *E:* dihydroxyephedrine; 4 cats. *F:* epinine; 6 cats. Increasing doses of the different substances were injected, the range of doses chosen being such as to give similar pressor effects for the different substances.

with the injection of 16 μg. noradrenaline which caused a mean contraction of 52 mm. in 25 cats. This smaller effect of corbasil on the nictitating membrane probably indicates a smaller affinity for the receptors on the membrane.

Adrenaline and neosynephrine were found to exert more effect on the normal membrane than noradrenaline or corbasil. Their effect on the denervated membrane was almost the same as that of noradrenaline.

DISCUSSION

The observations which have been described may be considered in the light of the evidence that the enzyme amine oxidase is present in the nictitating membrane (Robinson, 1952) and that this enzyme declines in amount after removal of the superior cervical ganglion (Burn and Robinson, 1952). The observations made from the third day onwards when further change is slight will be considered first. The substances of the first group, which have less than two -OH groups in the ring, and no -OH group in the side-chain, either fail to stimulate the nictitating membrane after denervation or stimulate it feebly. Those with least effect on the denervated membrane have no -OH group in the ring and a $-CH_3$ group on the α-carbon atom. These are amphetamine and its N-methyl derivative. The presence of one -OH group in the ring increases the action on the denervated membrane to some extent, and the removal of the $-CH_3$ group from the α-carbon atom to give tyramine increases it still more.

Since these substances have only a small action or very little on the denervated membrane, it is evident that their action on the normal membrane is likely to be connected with the presence of the transmitter at the nerve ending and its destruction by the enzyme system located there. The identification of this enzyme is supported by the findings with amphetamine, N-methylamphetamine, and its *p*-hydroxy derivative, since those substances which have a $-CH_3$ group on the α-carbon atom are known to inhibit amine oxidase. This suggests that the large effect of these substances on the normal membrane is indirect and is due to the action of the transmitter itself which, when the enzyme is inhibited, is no longer broken down.

This explanation, however, does not apply to tyramine and to β-phenylethylamine, which do not carry a $-CH_3$ on the α-carbon. They do not inhibit amine oxidase. Tyramine is actually a substrate which is more rapidly destroyed by this enzyme than noradrenaline. There is evidence (Bülbring, unpublished) that tyramine can potentiate the action of sympathetic stimulation and of adrenaline on the nictitating membrane. We can therefore suppose that tyramine competes with the transmitter for the enzyme and in this way increases the effect of the transmitter on the nictitating membrane. Thus, with tyramine and probably also with β-phenylethylamine, the mechanism of the contraction of the normal membrane is essentially similar to that already described for amphetamine, but the contraction is of short duration.

We have now to consider the change in the response to these substances at the end of 24 hours, when all stimulate the denervated membrane more than the normal membrane.

In line with the foregoing argument the action on the denervated membrane suggests that at this time some transmitter is still being liberated from the postganglionic fibres. Beyond this, however, any explanation is entirely speculative. Before the final death of the fibres there might be an increased liberation of transmitter or a fall in the amount of enzyme or both.

It remains to consider the increase in the sensitivity of the denervated membrane to low doses of *p*-hydroxy-N-methylamphetamine and especially to tyramine which persists for weeks in many cats.

The low dose of tyramine must stimulate the denervated membrane directly and its smaller effect on the normal membrane may be explained by its destruction by the greater amount of enzyme present there. However, the denervated membrane seems peculiar in that the extent to which it can be stimulated by tyramine is limited, and, as the dose is increased, no further effect is produced. With *p*-hydroxy-N-methylamphetamine there is also a permanent increase in the sensitivity of the denervated membrane to the lowest doses, though this is much less conspicuous than with tyramine. This cannot be explained by increased destruction of this substance in the normal membrane by the enzyme, since the enzyme does not destroy it. Presumably, however, since it inhibits the enzyme it combines with it, and therefore a greater proportion will be free to act on the denervated membrane where the amount of the enzyme is smaller.

Thus the consideration of the action of substances in the first group can be summarized by saying that the effects they produce in the normal and denervated membranes are all explicable in terms of the presence of an amount of amine oxidase in the normal membrane which declines in the denervated membrane and in terms of the weak direct stimulant action these substances possess.

The substances in the second group differed from those in the first in having an -OH group on the carbon atom next to the ring. All these substances had much more action on the denervated membrane than the substances in the first group. The action of the compounds in the second group resembled that in the first group in being greater on the denervated membrane during the first 24 hours after removal of the ganglion and in being less on the denervated membrane (except in 2 cats with ephedrine) thereafter. From the second day onwards their greater action on the normal membrane may be explained as the sum of the direct action on the membrane and the indirect action due to a diminished destruction of the transmitter when the substance either competes with the transmitter for the enzyme or inhibits the enzyme. The effect of the two compounds with a $-CH_3$ group on the α-carbon atom was more prolonged than that of the two compounds without this substituent. This is again consistent with the view that the enzyme concerned is amine oxidase.

The observation that β-phenylethanolamine and *p*-hydroxyphenylethanolamine acted for a longer time than β-phenylethylamine and tyramine respectively also accords satisfactorily with the biochemical evidence, since Blaschko (unpublished) found that the insertion of the -OH group in the side chain of tyramine slows down the rate of oxidation by amine oxidase.

The substances of the third group were with one exception catechol amines, having two -OH groups in the 3:4 position in the ring. Except for adrenaline and for neosynephrine (*m*-Sympatol) the effect of these compounds on the normal nictitating membrane was very small. When they were examined on the membrane 24 hours after removal of the ganglion, there was a small increase of sensitiveness ; when examined on the second and third day the increase was greater ; from the fourth day onward it became maximal and persisted.

These observations could be explained in terms of a diminution of amine oxidase only so far as the compounds without a $-CH_3$ group on the α-carbon atom were concerned. The substances corbasil and dihydroxyephedrine are not destroyed by amine oxidase, and yet they behave in the same way as noradrenaline and epinine. There are various possibilities. One is that the pathway of destruction of noradrenaline does not involve the enzyme amine oxidase and that some other mechanism is concerned which deals with all catechol amines. Another is that despite the similarity of the actions on the membranes of noradrenaline and of corbasil, they are destroyed in different ways, noradrenaline by amine oxidase and corbasil by another mechanism.

The curves in Fig. 7 which show parallelism for noradrenaline and corbasil but not for noradrenaline and adrenaline might be taken to mean that while the destruction of noradrenaline follows the same path as that of corbasil it follows a different path from the destruction of adrenaline. However, when Burn and Robinson (1952) published their observations on the effect of denervation on the amount of amine oxidase they found a close correlation between the D:N ratio (the ratio of the contraction of the denervated membrane to that of the normal membrane) for noradrenaline and that for adrenaline. We have now made similar observations in 25 more cats. In each the D:N ratio was calculated for 4 μg. and for 8 μg. doses of both noradrenaline and adrenaline. There was much variation in these ratios. Thus those for 8 μg. noradrenaline varied from 3.0 to 42.0. The D:N ratios for the two substances were, however, found to be significantly correlated (at the 5% level of significance) both for the 4 μg. dose and for the 8 μg. dose. Hence it appears that the factors which account for the increased sensitivity to noradrenaline are the same as those which account for the increased sensitivity to adrenaline, and from

this it can be argued that the pathway of destruction of noradrenaline is the same as that for adrenaline. We have not yet compared noradrenaline and corbasil in this way.

Recently Schayer (1951) has published results of studying the fate of adrenaline injected into rats when labelled with C^{14} in the N-methyl group and also when labelled on the β-carbon atom. He has come to the conclusion that " a reaction involving cleavage of the molecule at some point between the α-carbon and the methyl carbon is a major route of adrenaline metabolism." This supports the view that adrenaline is destroyed by amine oxidase, and therefore, from the preceding evidence of the correlation of the D:N ratios, it seems likely that noradrenaline is also destroyed in this way. Such a conclusion leaves the destruction of corbasil and of dihydroxyephedrine as an unsolved puzzle.

The phenomena observed with substances like amphetamine and tyramine of the first group, and with substances like ephedrine of the second group, appear to be satisfactorily explained in terms of amine oxidase. This implies that when noradrenaline is liberated from the nerve ending as transmitter it is destroyed by this enzyme. The conclusion does not follow, however, that when noradrenaline is injected it is destroyed in the same way. The possibility remains that all the dihydroxy compounds when injected are inactivated as a result of changes in the benzene ring.

When Barger and Dale (1910) introduced the conception of sympathomimetic amines, the part played by the chemical transmitter at the sympathetic nerve ending and by the enzyme located there to destroy it could not be considered. We can now say that it is probable that substances can produce sympathomimetic effects (*a*) directly by combining with receptors as the transmitter, noradrenaline, does; (*b*) indirectly by inhibiting or deviating the enzyme which destroys the transmitter; and (*c*) by a combination of both these direct and indirect actions. This distinction of three kinds of sympathomimetic action was clearly indicated in the discussion of the action of ephedrine by Gaddum and Kwiatkowski (1938). The results obtained by comparing the effects of various amines on the normal and denervated nictitating membranes, a method introduced by Bacq (1937), make it possible to say what structural features place a compound having two carbon atoms in the side chain in one or other of these groups.

Summary

1. A comparison has been made of the action of a number of sympathomimetic amines on the normal and denervated nictitating membranes of the spinal cat. Denervation consisted of removal of the superior cervical ganglion of one side, and observations were made at different times after the operation.

2. The amines tested could be classified by their action into three groups.

(*a*) Substances with not more than one -OH group in the benzene ring, and without an -OH group on the β-carbon atom, acted on the denervated membrane more powerfully than on the normal membrane only in the 24 hours following the operation. Later they caused little or no contraction of the denervated membrane. Their action appeared to be explained mainly by an inhibition or deviation of amine oxidase.

(*b*) Substances with not more than one -OH group in the benzene ring, but having an -OH group on the β-carbon atom, also acted on the denervated membrane more powerfully than on the normal membrane only in the 24 hours following the operation. Later they caused a considerable contraction of the denervated membrane but one which was smaller than that of the normal membrane. Their action appeared to be explained by a direct action on the membrane together with an inhibition or deviation of amine oxidase.

(*c*) Substances which were catechol amines had little action on the normal membrane and a much greater action on the denervated membrane from 48 hours after denervation onwards. Noradrenaline and corbasil were alike in their behaviour. The slight effect of these two substances on the normal membrane cannot be explained by supposing them to be rapidly destroyed by amine oxidase, since this enzyme does not attack corbasil.

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